

BONE DENSITY IN LIMB-IMMOBILIZED BEAGLES
AN ANIMAL MODEL FOR BONE LOSS IN WEIGHTLESSNESS

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Prolonged weightlessness in man in space flight results in a slow progressive demineralization of bone accompanied by an increased calcium output in the urine resulting in negative calcium balances. This possibly irreversible bone loss may constitute a serious limiting factor to long duration manned space flight. In order to seek and test preventative measures an appropriate ground-based animal model simulating weightlessness is necessary. Use of the mature Beagle in limb immobilization has been documented as an excellent model for orthopedic research since this animal most closely simulates the phenomenon of bone loss with regards to growth, remodelling, structure, chemistry and mineralization. The purpose of this project is to develop a research protocol for the study of bone loss in Beagles during and after (recovery) cast immobilization of a hindleg; research will then be initiated.

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INTRODUCTION

Loss of bone mineral in astronauts is a predictable major consequence of spaceflight (1). Mechanical unloading during weightlessness is the most obvious factor which may play a role in this phenomena since bone is dynamically responsive to the function demand placed on it (2). Two derangements of calcium metabolism occur during spaceflight: negative calcium balance and loss of bone mass. During 84 days spaceflight mineral content from astronauts' os calcis declined 4-5%, or about a loss of 100 mg calcium lost (3). There is some question whether this bone mineral loss is reversible post flight (4). Unchecked losses of body calcium and bone mass, coupled with normally-occurring age-related osteopenia, may eventually result in bone fracture, kidney stones or ectopic calcium deposition thereby compromising the health of crewmembers and mission success.

There is a need for a ground-based animal model for the study of the effect(s) of simulated weightlessness on bone loss. Several models have been proposed and evaluated by this laboratory (5). Experimentally induced immobilization bone loss in the beagle dog affords the best experimental model from the viewpoint of similarity of this specie's skeleton to that of man both in its static architecture and dynamic response(s) to environmental stresses.

This project will monitor the degree and rate of bone loss with limb hypodynamia and more importantly the degree and rate of possible bone recovery following reambulation.

OBJECTIVES

- 1) To immobilize right hindleg of mature beagles in order to simulate hypogravity or weightlessness for varying periods of time.
- 2) To measure regional and total bone loss over time in the immobilized limb, using dual-photon absorptiometry.
- 3) To measure time course and extent of possible bone recovery in the remobilized dog limb.
- 4) To monitor bone loss and possible recovery using histological and morphometrical techniques.

METHODS AND MATERIALS

The use of mature beagles in orthopedic research has several advantages over other animal models viz: the skeleton of adult man and the beagle are nearly identical in composition and manner of remodelling; well documented normative values for numerous physiological systems, including

bone histomorphometric data, are available; there are proven experimental models available for studies on specific types of bone loss; the time required for intervention studies is shorter than in man because of beagles' shorter life span and faster rate of bone turnover; beagles are available from commercial, inbred, controlled colonies (6-11).

Six 15-month old, young adult, male Beagles from a commercial closed inbred colony (Laboratory Research Enterprises, Kalamazoo, Mich.) will be used for this study. At this age the dogs are fully mature and all bone physes have closed. The dogs will be purchased free of endo- and ectoparasites and fully immunized, including rabies. They will have been partially muted and socialized. Each dog will have been marked by a distinctive ear tatoo and medical and genetic histories available from the supplier. They will be shipped by air freight and upon arrival be housed individually in the University of Houston laboratory animal facility in dog runs and examined by the staff veterinarian. The dog runs are temperature and humidity controlled and a 12 hr. light-dark cycle will be maintained. Each dog will be weighed shortly after arrival and at periodic intervals throughout the course of the experiment. They will be fed standard laboratory dog chow (Purina Canine Maintenance Diet) and top water ad libitum. The University of Houston animal facilities are fully accredited by A.A.A.L.A.C. and meet all N.I.H. standards.

The right hindleg of each animal will be immobilized in a nylon net pouch attached to a commercial dog jacket (Alice King Chatham Medical Arts, Los Angeles). The pouch will be closed by a zipper and adjusted by laces so that the lower hindleg containing the tibia is flexed back along the upper foreleg containing the femur. The result will be to shorten the leg so it cannot be used for ambulation. Bone density measurements (DP3 Dual Photon Spine Scanner, Lunar Radiation Corp., Madison, Wisc.) will be taken prior to immobilization and every 4 weeks thereafter. Trabecular bone of the distal femur and proximal tibia will be compared with shaft cortical regions. When bone loss is detected, at various times after immobilization, the hindleg will be remobilized by removing the constraining pouch and the dog allowed to ambulate freely on all four limbs. Bone density measurements will be taken periodically during the remobilization It period. It is anticipated that the extent of bone loss and the extent and speed of possible reversal after remobilization will be directly related to the duration of constraint and to the duration of remobilization, resp. While it is difficult to predict, bone loss in our young adult dogs may be seen as early as four weeks of constraint followed by possible bone loss reversal at about 8-10 weeks after mobilization (12,13). During bone density measurements the constraining pouch will be removed and the animals will be tranquilized with xylazine (Ropum) in order to insure the animal remains still during the procedures. At the end of the experiment animals will be sacrificed by an overdose of Nembutol. Bones of the control and experimental limbs will be used for histological and morphometry analyses including cross-sectional and medullary areas, measurements of resorption and oppositional rates (11).

ANTICIPATED OUTCOMES

Once a phenomenon is understood, at least in part, it can be manipulated, the aim of experimental therapeutics. Completion of these experiments will provide normative, base-line data on the extent and rate of bone loss during limb immobilization (employed as an analog of weightlessness) and, more critically, whether full bone recovery may be expected after remobilization. Our results will enable future studies on techniques for preventing bone loss in immobilization and promoting subsequent recovery. These techniques may include the effect of exercise, dietary calcium and/or phosphorus substances and the use of specific prophylactic drugs.

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